Our Field of Dreams

Team Emblazon

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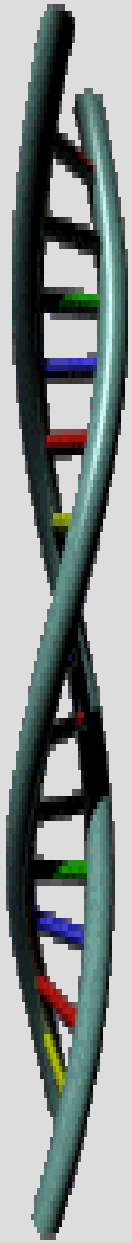
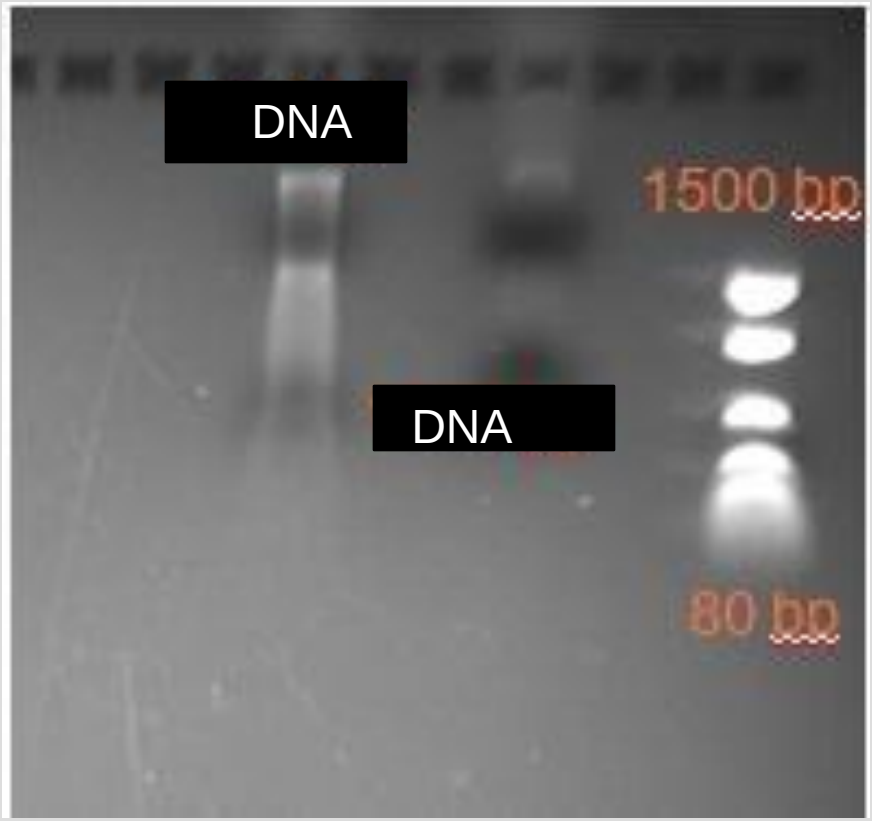


Original Plan

- Isolate DNA and RNA
  - Amplify using primers designed from mRNA sequence on GENBANK and that would add BioBrick prefix and suffix
- Sequence DNA and analyze for any introns
- Isolate vector and promoter from IGEM 2007 parts registry
- Insert new part and grow on naringenin enhanced media to produce blue colonies of *E. coli*

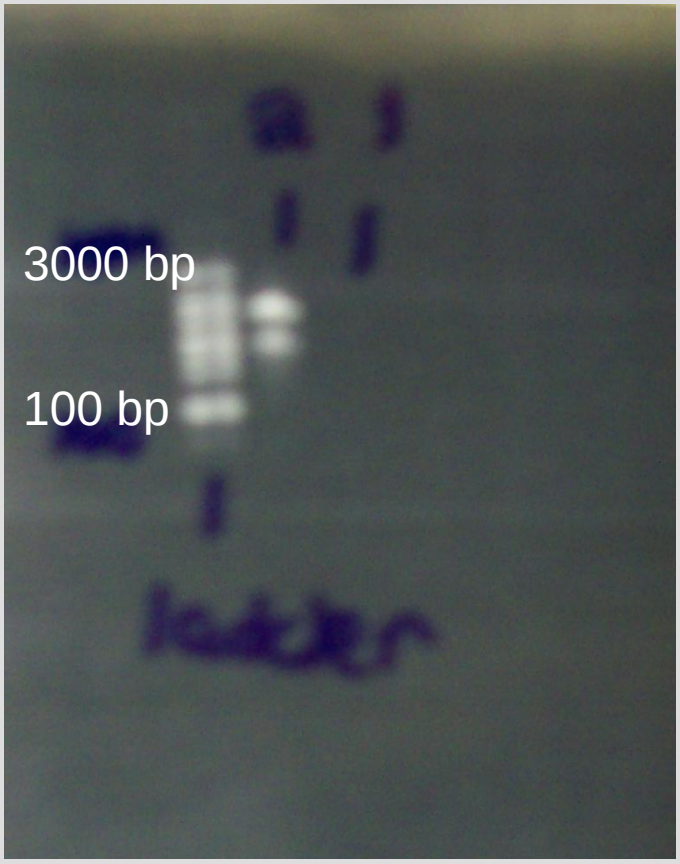
DNA Isolation

- Used Hops DNA Isolation protocol from Dr. Schwekendiek's lab



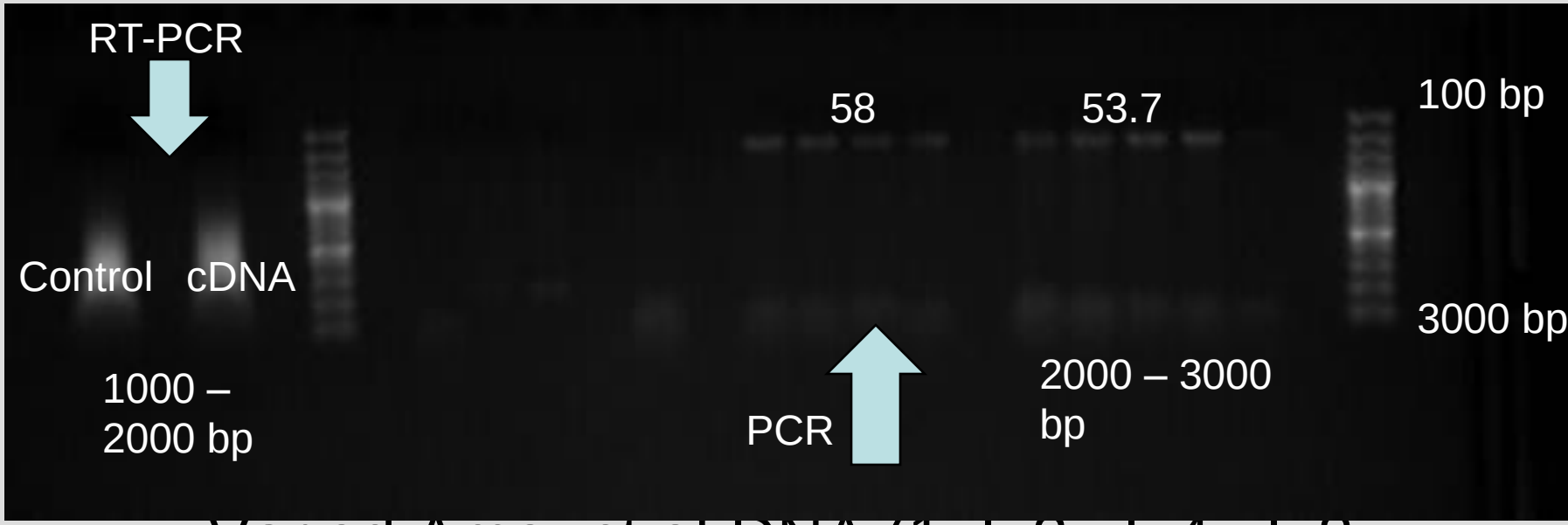
RNA Isolation

- Used Rneasy Kit from QIAGEN



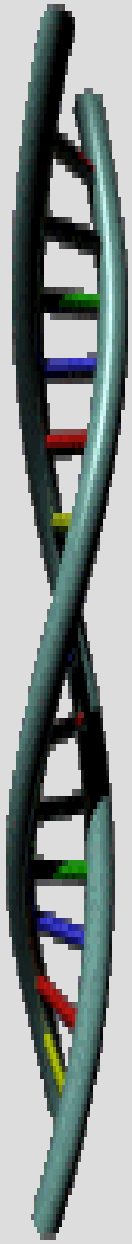
PCR Amplification

- Worked once, then failed repeatedly until it worked again



- Varied Amount of DNA (1µl, 2 µl, 4 µl, 8 µl, 10 µl) and temperature (53.7ºC and 58 ºC)

Gene Sequence



- >B01_p450-F_F1BBa_528838.Seq Sequence #0 of 7 downloaded on Fri Nov 21 11:40:23 CST 2008
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    CCCTTCATCGGCCCCATGCCCCACTCACGCCTCGCCCTCCTCTCCCGCCGCTACGGCCCCATC
    ATGTTCCTCAAAATGGGCATCCGCCAAGTGGTCGTCGCCTCCTCCTCCTCCGCCGCACGCTCC
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    CAACTTGCTGGTTTGTTCCATGGCGAATGTGATTGGGAGAATTGCAATGAGCAAGAGGGTGTTC
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    AACTTCCCCGCGCAAATCNTCCTCCCGGNGCCGAACGGTATGANTNAANATCGTNNCCCANNG
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    AANTCNNGCCNGTCTNANTCCACCTTNTATAACTCTANGAGAGTTATNANNNGANCTCACGCAA
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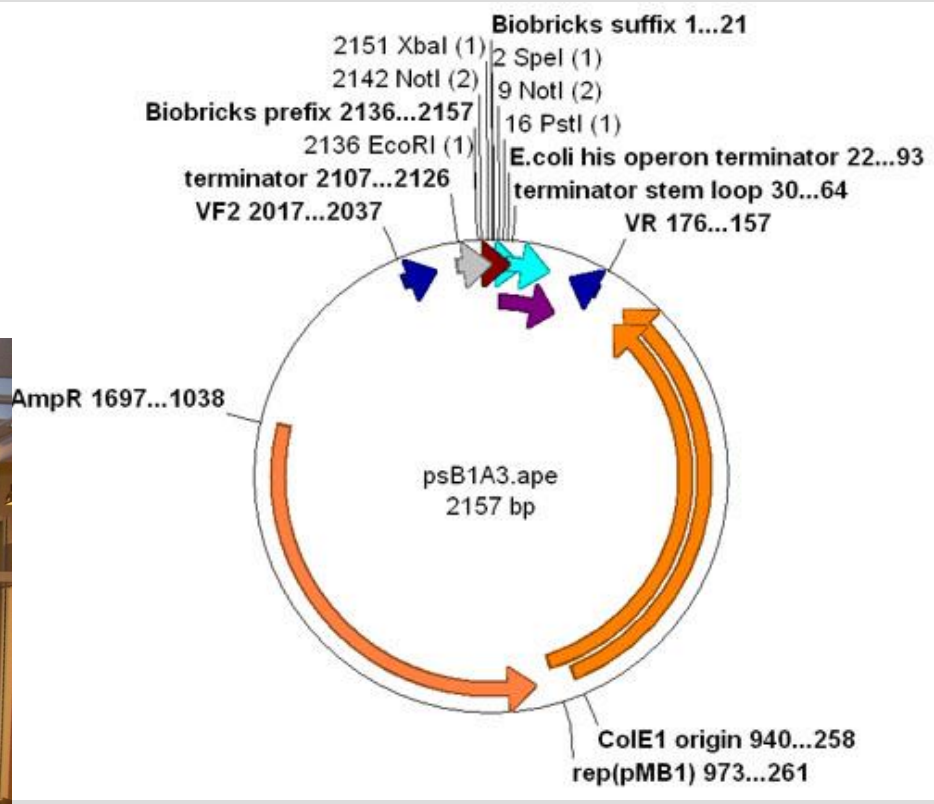
    AAANCAANNATTNTNNNNANCATTCNNTAATTAAANATATNCTCCNNCGCNNNNTTTCNAGANN
    NTNNCCNNNTNNNANNATTTCNTTANATTTTTTANNNAATANNNNTTTTTTNNTNNNN

BLAST Results

- Forward Read
  - 647 bp
  - 100% match to target sequence
- Reverse Read
  - 241 bp
  - 92% match to target sequence

pSB1A3 & pSB1A7

- High copy number plasmids carrying ampicillin resistance
- pSB1A3 is 2,157 bp
- pSB1A7 is 2, 431 bp



Verifying Plasmid Identity

- Isolate plasmid DNA
  - Glycerol stocks of *E. coli* DH5alpha
  - Miniprep/GET buffer protocol from Openwetware.org
- Restriction digest using fast digest EcoRI
- Electrophoresis
  - do not give the accurate result which should be 2,157 bp for pSB1A3 and 2,431 bp for pSB1A7
  - Lots of undigested DNA
- Repeated the experiment and same result

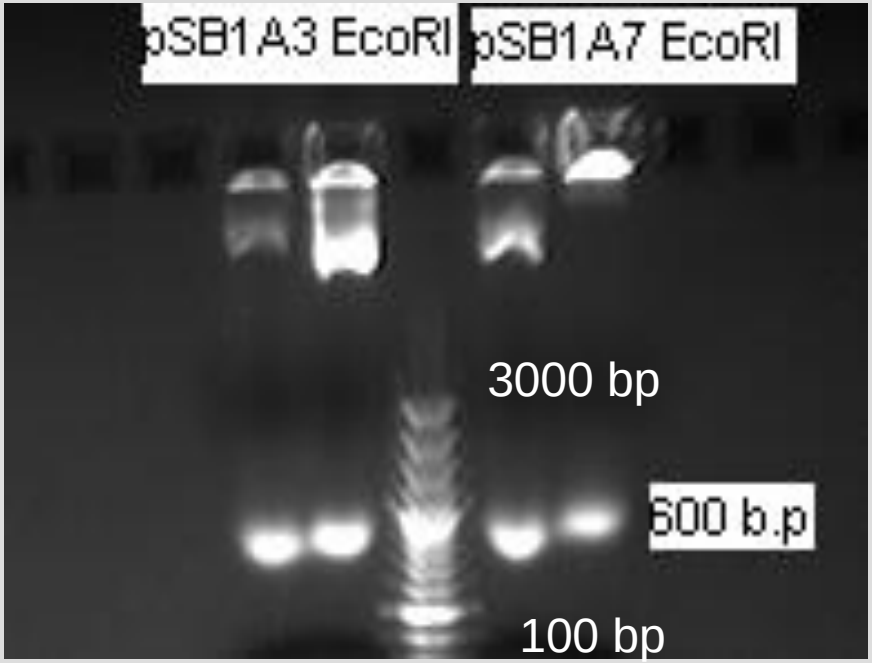
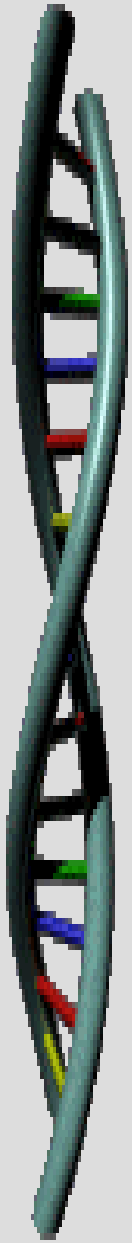


Fig. 1. Electrophoresis of pSB1A3 & pSB1A7 plasmid DNA digested with fast digest EcoRI

Other Extraction Attempts



- Extraction of plasmids from Parts Registry
  - We use registry kit of 2007 and paper punches from parts registry, spring 2008
  - Transform into NEB competent cell
  - Only the growth on control plates where we use pBLUE as a control
  - Parts not compatible with *E.coli* DH5alpha
  - Plasmids contain death gene called ccdB which kill all the competent NEB cells and occur no transformation.
- Extraction from different strain of *E. coli*, DB3.1
  - Digested with fast digest EcoRI, once again saw bands of incorrect size
  - But the control (pBLUE) works
  - Problem may be in the restriction enzyme we used, chemicals we prepared or in the plasmid itself
  - Repeat the same experiment, but different enzymes: fastdigest SpeI and Xbal and non fast digest EcoRI
  - Similar result
- Extraction from MIT samples
  - Digested with fast digest SpeI, fast digest XbaI, & non fast-digest EcoRI
  - pSB1A3 samples showed bands between 2,000 and 2,500 b.p
  - No clear bands appeared on the pSB1A7 samples

Procedure Change

- Used different plasmid extraction procedure, GeneJet Plasmid Miniprep Kit to extract plasmid DNA from MIT samples
  - used SpeI and XbaI for digestion
  - Clearer bands, much less undigested DNA
  - Band sizes were opposite
    - pSB1A3 showed band size of approximately 2,500 bp
    - pSB1A7 showed band size of approximately 2,100 bp

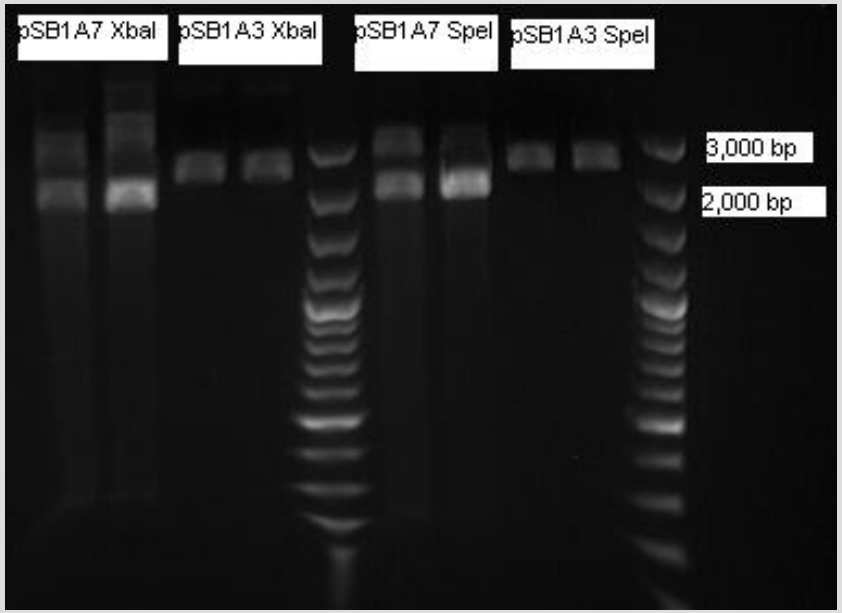


Fig. 2. Electrophoresis of pSB1A3 & pSB1A7 plasmid DNA digested with SpeI & XbaI after Gene Jet extraction procedure

Plasmid Verification using PCR

- PCR of pSB1A3 and pSB1A7 using VF2 and VR primers to amplify a segment of DNA
- VF2-VR= 316 bp in pSB1A3 and 590 bp in pSB1A7
- We observe VF2-VR is around 350 bp in pSB1A3 and around 900 bp in pSB1A7
- pSB1A7 is not target plasmid
- Also prove that the samples have been mislabeled while working in the laboratory only

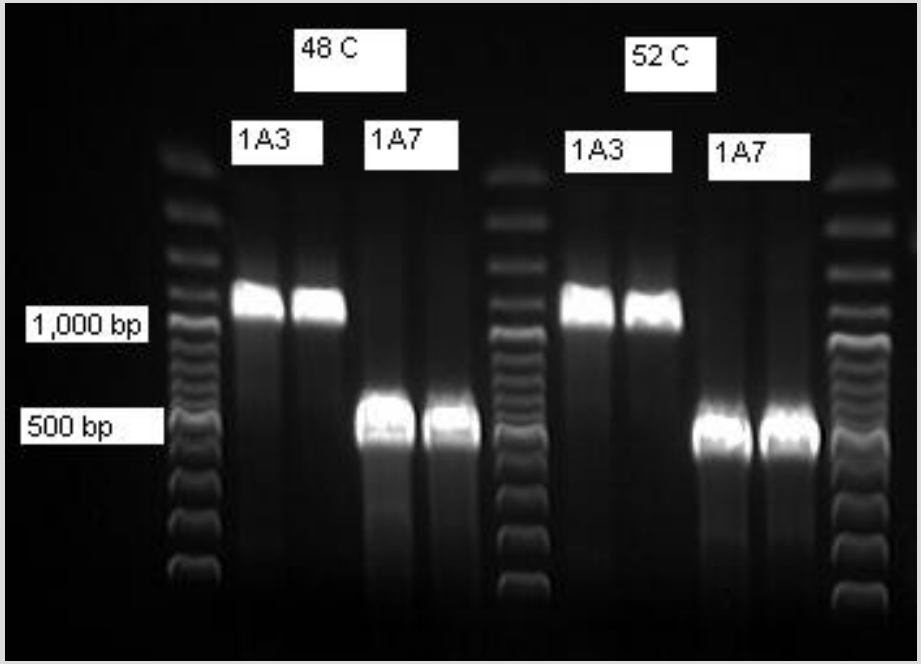


Fig. 3. Electrophoresis of amplified fragment of pSB1A3 & pSB1A7 after PCR using VF2 & VR primers at a temp range of 48C – 52C

Final Verification

- Extracted plasmid DNA using the Gene jet plasmid method
- Used SpeI and XbaI for digestion
- pSB1A3 showed bands at approximately 2,100 bp
- pSB1A7 showed bands at approx. 1,700 bp
- Concluded MIT samples of pSB1A7 contained “unknown” plasmid
- Also, MIT samples of pSB1A3 contained pSB1A3 plasmid described in registry

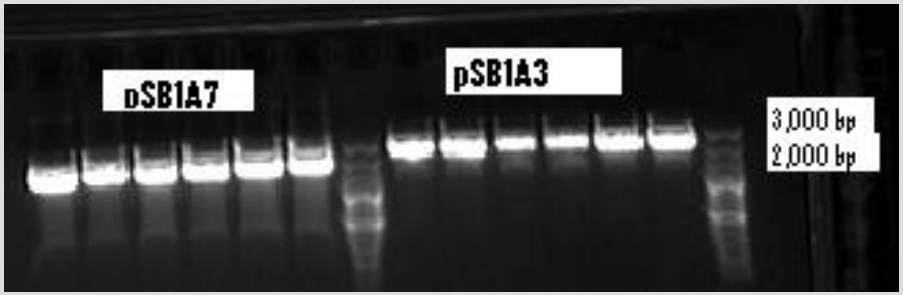
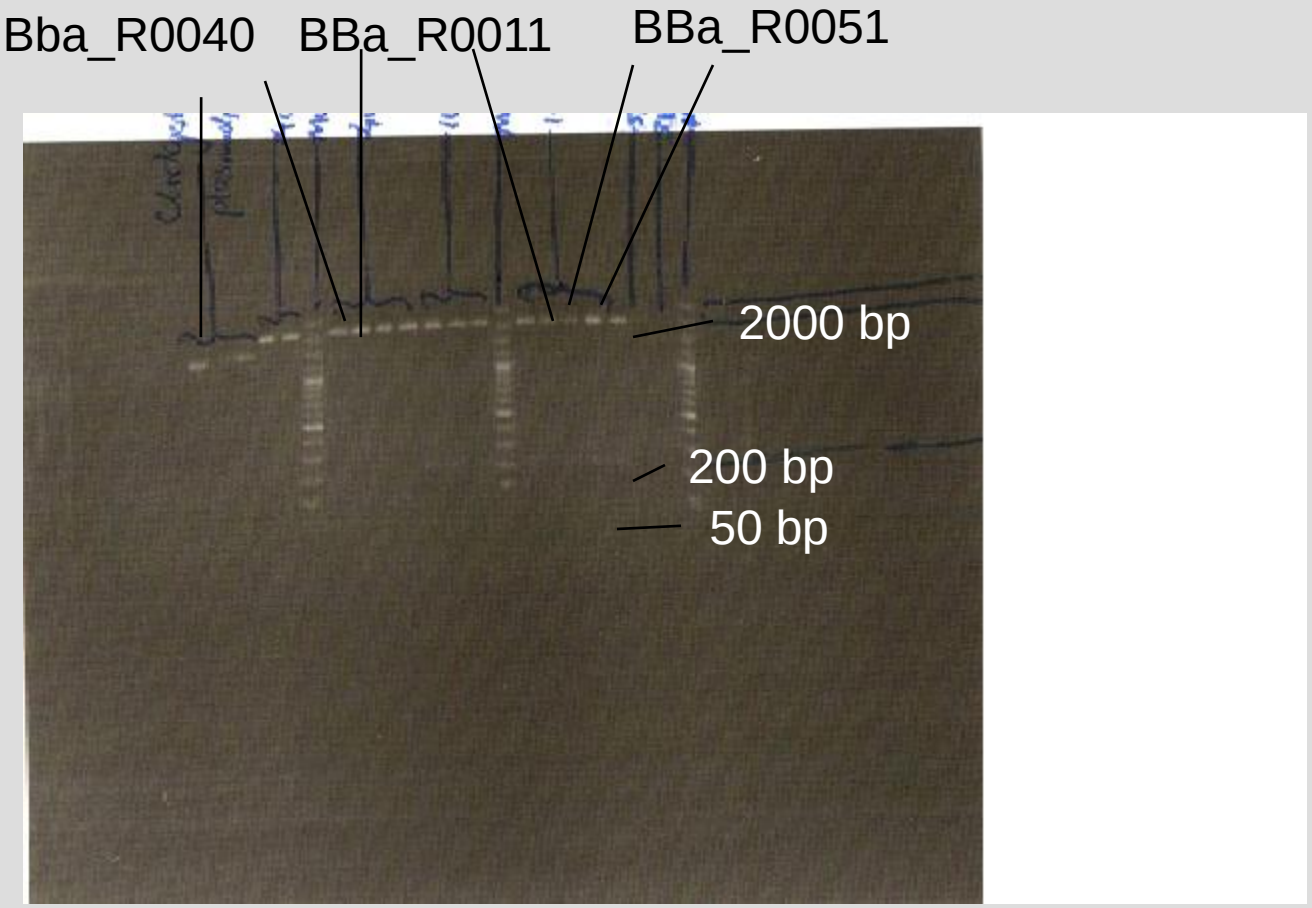


Fig. 3. Electrophoresis of pSB1A7 & pSB1A3 plasmid DNA samples digested with SpeI & XbaI

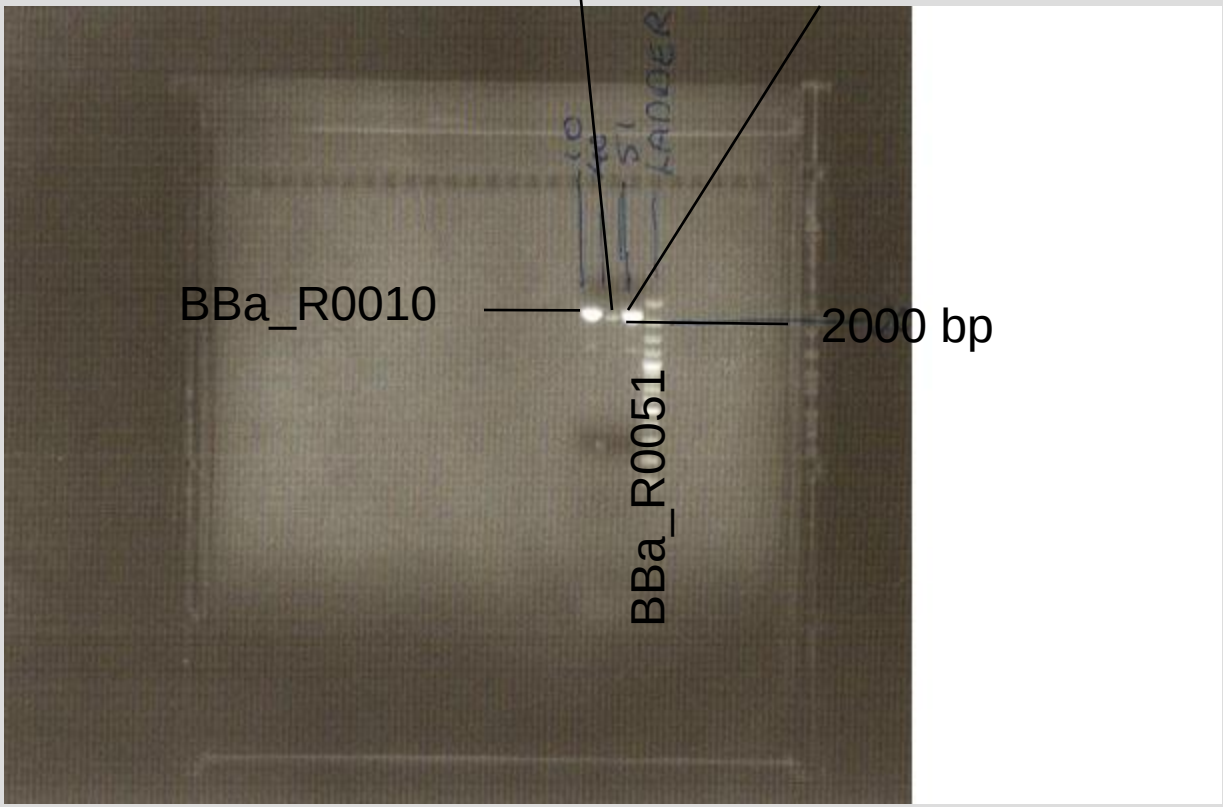
Selection of promoters

- We selected
  - BBa_R0040
  - BBa_R0051
  - BBa_R0010
  - BBa_R0011
  - BBa_I14032

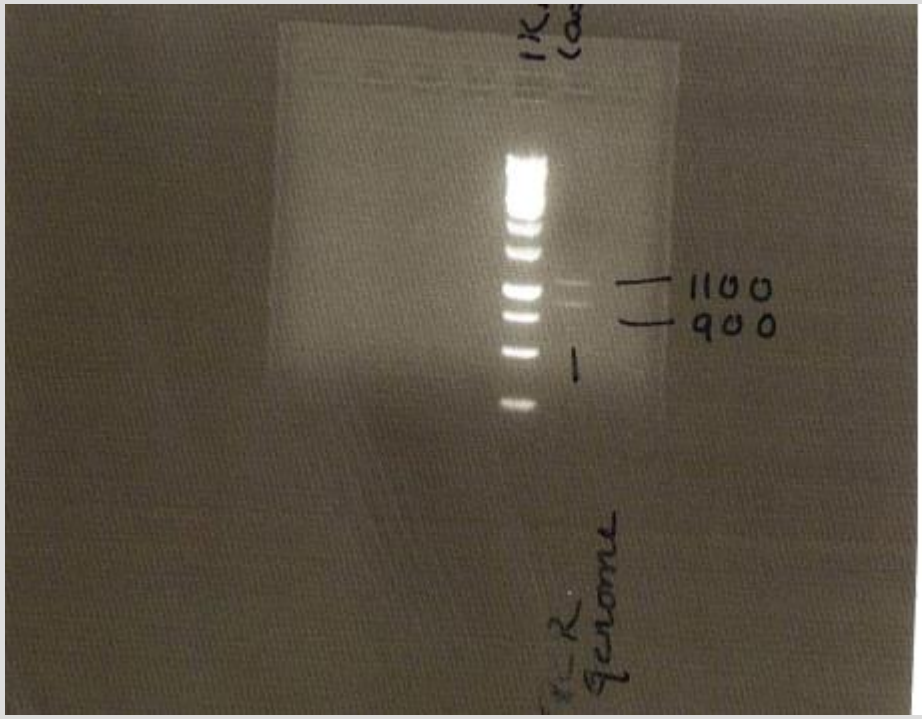
IDENTITY OF PROMOTERS



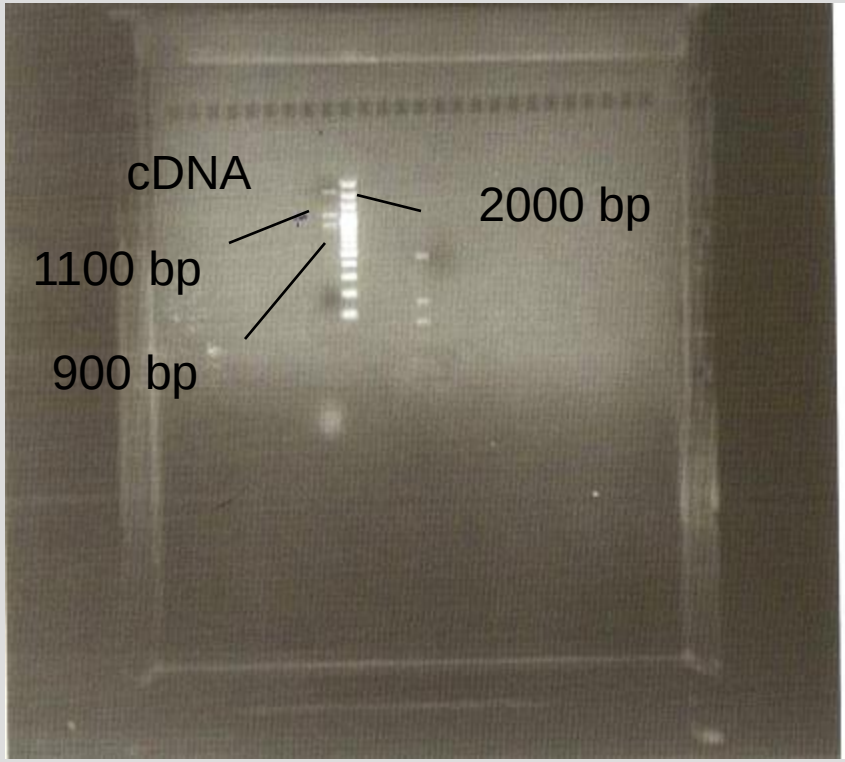
Double Digestion of pSB1A2 with Promoter with Spe1 & Pst1



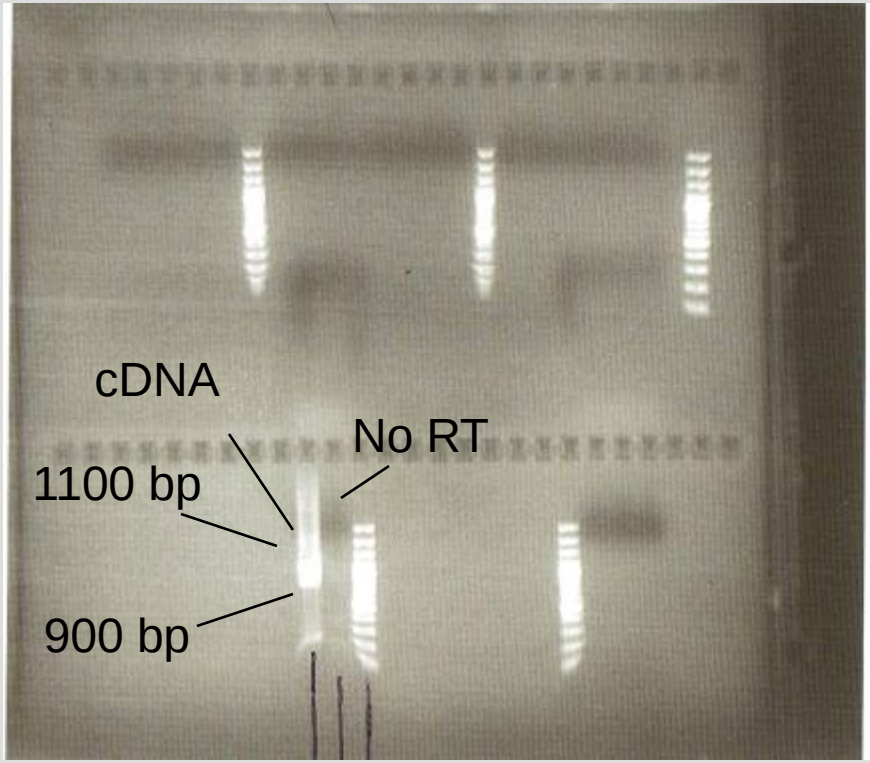
Double Digestion of PCR Product by Xba1 & Pst1

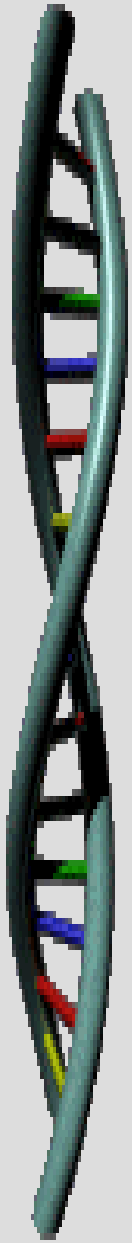


Double digestion of cDNA with Xba1 & Pst1



Double digestion of all RT PCR Samples with Xba1 & Pst1





Problems

- Missed a restriction site
  - Fix: Reanalyze mRNA sequence with more than one program
- PCR amplification
- Maintaining Stocks at Correct Temperatures
  - Fix: Make sure everyone reads the label

Special Thanks

Dr. Axel Schwekendiek

Our Financier

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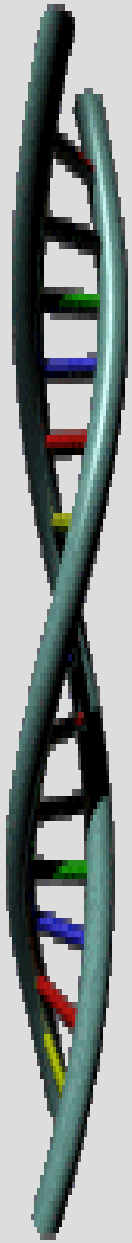
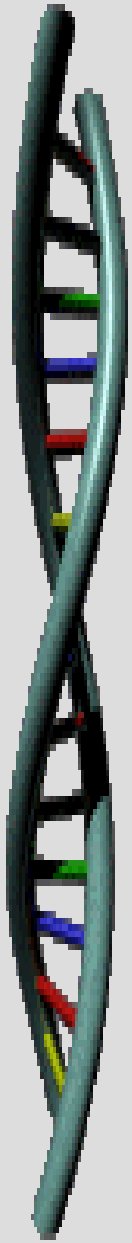


Remember

When all else fails try…

RING OF POWER!



How long your Prof. thinks it should take to do something		How long it'll actually take you to do it
"Trivial"	=	There goes your week.
"Easy enough"	=	Pull your hair out for a month.
"About a week"	=	Actually, this is pretty easy. He/she doesn't know there's technology that will do this for you now. Take the week off!
"Should keep you occupied for the rest of the term"	=	He/she will forget they asked you to do this by the end of the term. Don't even bother.
"This might make a good thesis topic"	=	Say hello to your thesis topic.
"Hmmm..."	=	Uh oh.